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Chronic Toxicity and Reproduction Studies of Hexachlorobutadiene in Rats

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Hexachlorobutadiene (HCBD), while not produced commercially in the United States, may be encountered as an unwanted by-product of certain processes associated with the chlorination of hydrocarbons. Studies were conducted to assess the potential long-term toxicity of HCBD. In a reproduction study conducted in rats, dose levels of 20 or 2.0 mg/kg-day of HCBD induced slight maternal toxicity (primarily of the kidney) but caused no adverse effects on reproductive parameters—percent pregnancy and neonatal survival/development. A decreased neonatal body weight was noted at the highest dose level of 20 mg/kg-day of HCBD. No toxicologic effects were observed among the adults at a dose level of 0.2 mg/kg-day or among the neonates at dose levels of 0.2 or 2.0 mg/kg-day of HCBD.

In a chronic toxicity study in rats, ingestion of 20 mg/kg-day for up to 2 years caused multiple toxicologic effects, primarily of the kidney, including the development of renal tubular adenomas and adenocarcinomas. Ingestion of the intermediate dose level of 2 mg/kg-day caused lesser degrees of toxicity, but no evidence of neoplasia. Ingestion of the lowest dose level of 0.2 mg/kg-day of HCBD caused no effects that could be attributed to treatment. These data indicate a dose-response relationship for HCBD-induced toxicity affecting primarily the kidney. HCBD-induced neoplasms occurred only at a dose level higher than that causing discernible renal injury.

Hexachlorobutadiene, $CCl_2=CCl-CCl=CCl_2$ (HCBD) is encountered as an unwanted by-product of certain processes associated with the chlorination of hydrocarbons. Although HCBD is not currently produced in the United States, HCBD reportedly has been used in Russia as a fumigant to treat grape phylloxera (*1*).

The acute toxicity data on HCBD have been reviewed (*2*). The acute oral LD_{50} has been reported to be 90 mg/kg for the guinea pig (*3*), 87–116 mg/kg for the mouse (*2, 4*) and 200–350 mg/kg for the rat (*2, 4*). HCBD was reported to be the most toxic of a series of chlorinated solvents in terms of acute oral lethality to mice (*5*).

Dermal application of 126 mg/kg of HCBD was lethal to ½ rabbits after 7 hr and 4/4 rabbits after 24 hr (2). All rabbits survived the dermal application of 120 mg/kg for 4 hr or 63 mg/kg for 24 hr. A report in the Russian literature suggested that neonatal rats may be more sensitive to the acute lethal effect of HCBD than adult rats. Deaths occurred among newborn of rats given a single subcutaneous dose of 20 mg HCBD/kg 3 months previously (*6*).

Single exposures to atmospheres containing 133–500 ppm HCBD for 4–7 hr caused the death of some or all rats exposed; all rats survived exposure to 161 ppm for 0.88 hr or 34 ppm for 3.5 hr (*2*). Most guinea pigs and cats died subsequent to exposure to 161 ppm for 0.88 hr or 34 ppm for 7.5 hr (*2*). In short-term repeated inhalation studies with HCBD (*7*), daily 6-hr exposures to 25 ppm HCBD caused respiratory difficulty, decreased weight gain and pathologic injury to the tubular epithelium of the kidneys; 12 daily 6-hr exposures to 100 ppm HCBD caused more severe toxicity, including the death of some rats. There was no evidence of toxicity with 15 daily 6-hr exposures to 10–5 ppm, except for

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retarded weight gain in female rats exposed to 10 ppm of HCBD. Another group (*4*) reported that exposure to 0.024 mg HCBD/l. air (2.3 ppm) for 7 months (number of hours/day or number of days/week not given) caused no alterations in mice or rats.

In a previous 30-day toxicity study in our laboratory, rats received HCBD in the diet at daily dose levels ranging from 1 to 100 mg/kg (*8*). Renal toxicity in the form of an increase in the kidney-body weight ratio as well as renal tubular degeneration, necrosis and regeneration occurred in rats receiving 30, 65 and 100 mg HCBD/kg-day. Other adverse effects observed included decreased food consumption and body weight gain at 10, 30, 65, and 100 mg/kg-day, minimal hepatocellular swelling at 100 mg/kg-day and hemoconcentration at 10, 30, 65, and 100 mg/kg-day. No adverse effects were observed among those receiving 3 mg/kg-day. The kidney was the organ most sensitive to the deleterious effects of HCBD. In a reproduction study (*9*) conducted in our laboratory, maintenance of adult Japanese quail for a period of 90 days on diets containing HCBD concentrations up to 30 ppm (about 5 mg/kg-day) had no deleterious effect on reproduction.

Based on the above review of the available toxicity data on HCBD, it became apparent that there was a need for data to more adequately assess the potential toxicity of HCBD, especially in regard to chronic toxicity. Thus, our laboratory conducted additional toxicity studies with HCBD. The results of one of these studies dealing with the lifetime ingestion by rats of diets containing HCBD has been reported previously in detail (*10*). The results of a reproduction study on rats ingesting diets containing HCBD has also been reported in detail (*11*).

This paper herein reviews the highlights of our studies reported previously in detail (*10, 11*) as well as some additional data on the acute oral lethality of HCBD in neonatal versus adult rats.

Materials and Methods

Sample of HCBD

The sample of HCBD used in these toxicity studies was supplied by the Dow Chemical Company, Midland, Michigan, and was described as 99.00% HCBD.

Acute Lethality Study in Rats

HCBD was given as a corn oil solution by oral gavage to Sprague-Dawley neonatal rats (21–22 days of age) and adult rats. The test solution was prepared so that a volume of 10 ml/kg provided the desired dose of HCBD. There were five to six rats/dose level. The rats were observed until they were no longer showing signs of toxicity. The LD_{50} value was calculated according to a published procedure (*12*).

Reproduction Study in Rats

The details of this study have been given in the previously cited paper (*11*). Groups of male and female Sprague-Dawley rats were maintained on diets supplying 0, 20, 2, or 0.2 mg/kg-day of HCBD for 90 days prior to mating, 15 days during mating, and, subsequently, throughout gestation and lactation. After 21 days of lactation, the dams and their young were killed and given a gross pathologic examination. Various organs from each adult rat were weighed and microscopic examination was conducted on tissues for all organ systems. Preterminal hematologic and urinary analyses were conducted on adult males and females. Terminal serum samples were collected from the adult males and females for determination of blood urea nitrogen (BUN), creatinine, and serum glutamic pyruvic transaminase (SGPT) activity.

The reproductive parameters assessed in this study are listed in Table 3 below. In addition, the weanlings of each litter were subjected to a gross autopsy examination, with microscopic examination of liver, kidney, spleen, heart, lung and testes from males and females on the control and top dose levels. Statistical evaluation of reproductive indices were made by the Fisher's Exact Probability Test (*13*). Body organ weight data were analyzed by an analysis of variance and Dunnett's Test (*14*). Level of significance was $p < 0.05$.

Chronic Dietary Study in Rats

In this study, male and female Sprague-Dawley rats were maintained on diets containing HCBD for the duration of their lifetime. Specific details have been given in the previously cited publication (*10*). The rats were maintained on diets supplying 0, 20, 2.0, or 0.2 mg HCBD/kg-day for up to 24 months. Group sizes were 39–40 rats/sex/dose level plus 90/sex for controls. Parameters monitored at various intervals included appearance and demeanor, body weights, food consumption, hematologic and urine analyses, urinary prophyrins and serum clinical chemistry (BUN), serum alkaline phosphatase (AP) and SGPT activity. All rats dying or culled during the study were subjected to gross pathologic examination, with subsequent microscopic examination on tissues from all the rats.

Terminal necropsy examination (after 22 months for males, 24 months for females) included determi-

nation of the weights of brain, heart, liver, kidneys, and testes plus gross and microscopic examination of tissues of all rats. Resultant data were analyzed statistically by an analysis of variance and Dunnett's Test (*14*), except for tumor data, which were analyzed with Fisher's Exact Probability Test (*13*). Level of significance was $p < 0.05$.

Results

The results of the acute oral lethality study are listed in Table 1. The LD_{50} of HCBD in adult male and female rats following a single oral dose was 580 (504–667) mg/kg and 200–400 mg/kg, respectively. The LD_{50} in 21-day-old male and female weanling rats was 64 (46–91) mg/kg and 46 (26–81) mg/kg, respectively. The majority of the deaths occurred on the second or third day after dosage with some deaths as late as 17 days after dosage.

Table 1. Results of acute oral lethality study of HCBD in weanling and adult rats.

	LD_{50} + 95% confidence limits, mg/kg	
Age of rats	Male	Female
Adult	580 (504–667)	200–400 (LD_{50} and confidence limits not calculable)
21-22 days	65 (46–91)	46 (26–81)

Reproduction Study in Rats

Detailed results have been included in the paper cited previously (*11*). These results are summarized in Tables 2 and 3 on the basis of comparison with control data. Adult male and female rats ingesting the high dose level of 20 mg/kg-day of HCBD showed multiple toxicologic effects, including decreases in food consumption and body weight gain. Terminal kidney weights of these males and females were increased, as were the liver weights of the males. Pathologic examination revealed renal tubular epithelial degeneration and regeneration in both males and females ingesting 20 mg/kg-day of HCBD. Adult rats ingesting the intermediate dose level of 2 mg/kg-day of HCBD were comparable to controls, except for a much lower incidence of the renal tubular histopathologic changes noted at the high dose level. Adult rats ingesting 0.2 mg/kg-day of HCBD had no observations considered related to treatment.

The parameters used to assess the reproductive performance of the rats are summarized in Table 3. The ingestion of 20, 2, or 0.2 mg/kg-day of HCBD had no effect on the percent pregnancy, gestation survival, neonatal survival, neonatal sex ratio or morphologic alterations in neonates. Neonatal body weights were unaffected except for a decreased body weight on day 21 for the neonates of the high dose level, 20 mg/kg-day of HCBD.

Results of Chronic Dietary Study in Rats

Detailed data on the results of the study have been included in the publication cited previously (*10*). These results are summarized in Table 4. Lifetime ingestion of the highest dose level of 20 mg/kg-day of HCBD caused multiple toxicologic effects. This included increased mortality (males), decreased body weight gain (males and females), increased urinary excretion of coproporphyrin (males and females), and increased terminal weights

Table 2. Observations on adult rats used in reproduction study with HCBD (interpretation comparative to control data).

	Comparison with controls[a]					
	HCBD 20 mg/kg-day		HCBD 2 mg/kg-day		HCBD 0.2 mg/kg-day	
Parameters	M	F	M	F	M	F
Clinical observations	—	—	—	—	—	—
Food consumption	DEC	DEC	—	—	—	—
Body weight gain	DEC	DEC	—	—	—	—
Clinical chemistry						
BUN	DEC*	—	DEC*	—	—	—
SGPT	—	—	—	—	—	—
Creatinine	—	—	—	—	—	—
Terminal organ weights						
Kidney	INC	INC	—	—	—	—
Liver	INC	—	—	—	—	—
Pathology examination	Renal tubular degeneration and regeneration		Low incidence of same		—	—

[a]Code: (—) comparable to control data; (*) considered to be of questionable toxicologic significance; (INC) or (DEC) indicates parameter was increased or decreased relative to control data.

Table 3. Observations on reproductive parameters in study with HCBD in rats (interpretation comparative to control data).

Reproductive parameters	Comparison with controls[a]		
	HCBD 20 mg/kg-day	HCBD 2 mg/kg-day	HCBD 0.2 mg/kg-day
Pregnancy rate (%)	—	—	—
Gestation survival	—	—	—
24 hr survival	—	—	—
7-Day survival	—	—	—
14-Day survival	—	—	—
21-Day survival	—	—	—
Neonatal body weight			
Day 1	—	—	—
Day 7	—	—	—
Day 14	—	—	—
Day 21	DEC	—	—
Sex Ratio M/F	—	—	—
Gross autopsy examination of weanlings	—	—	—
Histopathological examination of weanlings	—	Not examined	

[a]See Table 2.

Table 4. Results of chronic dietary study with HCBD in rats (interpretation comparative to control data).

Parameter	Comparison with control[a]					
	HCBD 20 mg/kg-day		HCBD 2 mg/kg-day		HCBD 0.2 mg/kg-day	
	M	F	M	F	M	F
Mortality	INC	—	—	—	—	—
Body weight gain	DEC	DEC	—	—	—	—
Food consumption	—	—	—	—	—	—
Hematology (RBC) (others unaffected)	DEC[b]	—	—	—	—	—
Urinalysis						
Urine concentration test	—	—	—	—	—	—
Urinary coproporphyrin (others unaffected)	INC	INC	—	INC	—	—
Clinical chemistry						
BUN	—	—	—	—	—	—
SGPT	—	—	—	—	—	—
SAP	—	—	—	—	—	—
Terminal organ weights, kidneys	INC	INC	—	—	—	—
Autopsy examination	Kidney nodules		—	—	—	—
Histopathological examination	Kidney neoplasia		—	—	—	—
	Kidney hyperplasia		Kidney hyperplasia		—	—

[a]See Table 2.
[b]Possible secondary to renal toxicity.

Table 5. Renal tubular neoplasms in chronic dietary study with HCBD in rats.

HCBD dose levels, mg/kg-day	Sex	Renal tubular adenoma		Renal tubular adenocarcinoma		Total	
		Number of rats	% of rats	Number of rats	% of rats	Number of rats	% of rats
0	M	1/90	1.7	0/90	0	1/90	1.1
20		2/39	5.1	9/39	18	9/39	23.1[a]
2.0		0/40	0	0/40	0	0/40	0
0.2		0/40	0	0/40	0	0/40	0
0	F	0/90	0	0/90	0	0/90	0
20		3/40	7.5	3/40	7.5	0/40	15[a]
0.2		0/40	0	0/40	0	0/40	0
0.2		0/40	0	0/40	0	0/40	0

[a]$p < 0.05$.

of kidneys (males and females).

Pathologic examination revealed changes in the kidneys, including hyperplasia and neoplasia of renal tubular epithelium. Some of the neoplasms were noted grossly as nodules in the kidneys. These nodules in the kidneys were microscopically diagnosed as renal tubular adenomas or adenocarcinomas, some of which metastasized to the lungs. Approximately 23% of the males and 15% of the females from the 20 mg/kg-day dose level had renal tubular neoplasms (Table 5).

At the intermediate dose level of 2.0 mg/kg-day of HCBD, findings considered related to treatment were limited to an increased urinary excretion of coproporphyrin (females only) and increased hyperplasia of renal tubular epithelium. This intermediate dose level of 2 mg/kg-day of HCBD caused no neoplasms considered related to treatment. Lifetime ingestion of the lowest dose level of 0.2 mg/kg-day of HCBD caused no discernible ill effects in any of the parameters monitored in this study.

Discussion

These recent studies reveal several pertinent observations on the toxicity of HCBD. On an acute basis, the weanling rat appears to be substantially more sensitive than the adult rat to a single oral dose of HCBD.

In the reproduction study, dose levels of 20 or 2.0 mg/kg-day of HCBD induced maternal toxicity (primarily of the kidney) but caused no adverse effects on reproductive parameters—percent pregnancy and neonatal survival/development. A decreased neonatal body weight on day 21 was noted only at the highest dose level of 20 mg/kg-day of HCBD. No toxicological effects were observed among the adults at a dose level of 0.2 mg/kg-day or among the neonates at dose levels of 0.2 or 2.0 mg/kg-day of HCBD.

In the chronic dietary study, as in shorter-term studies conducted previously with HCBD, the kidney appeared to be the primary target organ. Ingestion of 20 mg/kg-day of HCBD for up to 2 years caused multiple toxicologic effects, including decreased body weight gain, increased mortality, increased urinary excretion of coproporphyrin, increased weights of kidneys, increased renal tubular epithelial hyperplasia, and renal tubular adenomas and adenocarcinomas, some of which metastasized to the lungs.

Ingestion of the intermediate dose level of 2 mg/kg-day of HCBD for up to 2 years caused lesser degrees of toxicity, including an increase in urinary coproporphyrin excretion and an increase in renal tubular epithelial hyperplasia. The fact that urinary excretion of coproporphyrin was increased at this dose level which produced no neoplasms may indicate the usefulness of this parameter as a biological monitor when included in a medical surveillance program for workers exposed to HCBD.

Ingestion of the lowest level dose of 0.2 mg/kg-day of HCBD for up to 2 years caused no effects that could be attributed to treatment. Whereas the intermediate dose level of 2.0 mg/kg-day caused a slight degree of renal toxicity, the highest dose level of 20 mg/kg-day for up to 2 years caused multiple and substantial toxicologic effects, including renal tubular neoplasms. Thus, these data indicate a clear-cut dose-response relationship for HCBD-induced toxicity affecting primarily the kidney. HCBD-induced renal neoplasms occurred only at a dose level higher than that causing discernible renal injury.

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